Glycogen Content of Adipose Tissue, Heart and Liver of
AEM Control, Flight and Hindlimb-suspended Animals

Group	Glycogen		
	Adipose nmol glucose/mg tissue	Heart nmol glucose/mg protein	Liver μmol glucose/mg protein
AEM Control	1.04 ± 0.09	42.4 ± 2.2	1.06 ± 0.05
Flight	0.57 ± 0.05[a]	58.7 ± 1.8[a]	1.45 ± 0.07[a]
Suspended	1.51 ± 0.15	43.3 ± 2.4	0.98 ± 0.07

[a]$p<0.01$ versus AEM control by ANOVA with Bonferroni correction

AEM control animals were housed in the Animal Enclosure Module at the University of Arizona animal facility under the same temperature conditions, light-dark cycle, and duration as flight animals (Tischler et al J. Appl. Physiol. 74:2161, 1993). Flight animals were randomly assigned and loaded into their AEM at 2300 h EDT, September 11, 1991 (launch-21h). The final flight values were determined, between 2h 8 min and 3h 22 min after landing. Suspended animals were selected from the same shipment as the AEM control group.

Glycogen was determined by dissolving the tissue in 1 ml of 5N KOH by heating in boiling water in 12 ml conical tubes. The 0.2 ml saturated Na_2SO_4 was added followed by 1.5 ml 95% ethanol. The solution was boiled an additional 2 minutes and then cooled on ice. The solution was then centrifuged at 2500 x g for 10 min. The pellet was dissolved again in 1 ml of 5N KOH by heating in a boiling water bath followed by addition of 1.5 ml 95% ethanol with further boiling for 2 minutes. After cooling on ice the solution was centrifuged as above. The pellet was stored at -20^{o}C overnight. The next day 0.5 ml of 2N HCl was added to the pellet and was incubated in a boiling water bath for 150 minutes. After cooling on ice, the solution was neutralized with 4 N KOH,).1 M triethanolamine to between pH 6 and 8. The solution was then brought to a final volume of 1 ml with distilled water.

Glucose in the samples was analyzed spectrophotmetrically by following the formation of NADPH at 34o nm using the coupled reactions of hexokinase (glucose + ATP → glucose-6-phosphate + ADP) and glucose-6-phosphate dehydrogenase (glucose-6-phosphate + $NADP^+$ → 6-phosphogluconate + NADPH). The buffer mixture contained in 10 ml 80 mM triethanolamine (pH 7.4), 16 mM $MgCl_2$, 8 mM ethylenediaminetetracetic acid (pH 7.4), 6.1 mg ARP, 8 mg $NADP^+$, and 3.5 units glucose-6-phosphate dehydrogenase. To 0.5 ml of buffer mixture was added processed tissue sample plus water at a final volume of 0.5 ml. The amount of processed samples used were: adipose, 0.5 ml; heart from flight animals , 0.05 ml; heart from AEM control and suspended animals, 0.1 ml; liver from flight and suspended animals, 0.25 ml diluted 100-fold; liver from AEM control and suspended animals, 0.50 ml diluted 100-fold. After taking an initial reading at 340 nm, hexokinase was added to initiate the reaction and a final reading was taken. Glucose in the analyzed sample was proportionate to the amount of NADPH produced calculated using an extinction coefficient of 6.22 mM^{-1}.